

Serial No. 09/545,334
Group Art Unit: 1635

Attorney Docket No. 0803

**COMPLETE SET OF CLAIMS AS
MODIFIED BY CURRENT AMENDMENT**

WHAT IS CLAIMED IS:

1. A method for producing fertile, transgenic plants capable of the regulated expression of a cytokinin modulating gene in developing seeds comprising:
introducing into plant host cells a genetic construct capable of preferential temporal and/or spatial expression of a cytokinin modulating gene in developing seed under conditions sufficient for the stable integration of the construct into the genome of said cells; and
regenerating and recovering said fertile transgenic plants.
2. The method according to Claim 1 wherein the introduction of said construct is carried out by a process selected from the group consisting of electroporation, PEG poration, particle bombardment, silicon fiber delivery, microinjection, and Agrobacterium-mediated transformation.
3. The method according to Claim 2 wherein said process of introduction is particle bombardment.
4. The method according to Claim 2 wherein said process of introduction is Agrobacterium-mediated transformation.
5. The method according to Claim 1 wherein said genetic construct comprises a promoter directing temporal and/or spatial gene expression in plant seed operatively linked to a cytokinin modulating gene.
6. (Canceled)
7. The method according to Claim 5 wherein said seed is from a monocotyledonous plant and said promoter is selected from the group consisting of maize 15KD zein, 22KD zein, 27KD gamma zein, waxy, shrunken-1, shrunken -2, globulin-1, cim-1, end1, end 2, and gzw64a, and barley ltp2.
8. The method according to Claim 5 wherein said promoter directs embryo-preferred expression.
9. The method according to Claim 8 wherein said promoter is globulin-1.
10. The method according to Claim 5 wherein said promoter directs endosperm-preferred expression.
11. The method according to Claim 10 wherein said promoter is 27KD gamma zein.
12. (Canceled)

Serial No. 09/545,334
Group Art Unit: 1635

Attorney Docket No. 0803

13. (Amended) The method according to Claim 1 wherein said modulating gene encodes a cytokinin biosynthetic enzyme.
14. The method according to Claim 13 wherein said modulating gene encodes isopentenyl tranferase.
15. (Canceled)
16. (Canceled)
17. A fertile transgenic plant comprising a genetic construct stably integrated into the genome thereof, said construct capable of the temporal and/or spatial modulation of cytokinin levels in developing seed of said plant.
18. The plant according to Claim 17 wherein said genetic construct comprises a promoter directing temporal and/or spatial gene expression in plant seed operatively linked to a cytokinin modulating gene.
19. (Canceled)
20. The plant according to Claim 18 wherein said seed is from a monocotyledonous plant and said promoter is selected from the group consisting of maize 15KD zein, 22KD zein, 27KD gamma zein, waxy, shrunken-1, shrunken -2, globulin-1, cim-1, end1, end2, and gzw64a, and barley ltp2.
21. The plant according to Claim 18 wherein said promoter directs embryo-preferred expression.
22. The plant according to Claim 21 wherein said promoter is globulin-1.
23. The plant according to Claim 18 wherein said promoter directs endosperm-preferred expression.
24. The plant according to Claim 23 wherein said promoter is 27KD gamma zein.
25. (Canceled)
26. (Amended) The plant according to Claim 18 wherein said modulating gene encodes a cytokinin biosynthetic enzyme.
27. The plant according to Claim 26 wherein said modulating gene encodes isopentenyl tranferase.
28. (Canceled)
29. (Canceled)
30. An isolated recombinant DNA comprising a genetic construct that comprises a promoter directing temporal and/or spatial gene expression in plant seed operatively linked to a cytokinin modulating gene.
31. (Canceled)

Serial No. 09/545,334
Group Art Unit: 1635

Attorney Docket No. 0803

32. The DNA according to Claim 30 wherein said seed is from a monocotyledonous plant and said promoter is selected from the group consisting of maize 15KD zein, 22KD zein, 27KD gamma zein, waxy, shrunken-1, shrunken-2, globulin-1, cim-1, end1, end2, and gzw64a, and barley ltp2.
33. The DNA according to Claim 30 wherein said promoter directs embryo-preferred expression.
34. The DNA according to Claim 33 wherein said promoter is globulin-1.
35. The DNA according to Claim 30 wherein said promoter directs endosperm-preferred expression.
36. The DNA according to Claim 35 wherein said promoter is 27KD gamma zein.
37. (Canceled)
38. (Amended) The DNA according to Claim 30 wherein said modulating gene encodes a cytokinin biosynthetic enzyme.
39. The DNA according to Claim 38 wherein said modulating gene encodes Isopentenyl transferase.
40. (Canceled)
41. (Canceled)
42. Host plant cells having stably introduced therein the genetic construct of Claim 30.
43. A method for improving stress tolerance and yield stability in plants in need thereof comprising stably introducing into cells of said plants a genetic construct capable of preferentially expressing cytokinin modulating genes during the lag phase of plant seed development and regenerating and recovering plants from said cells.
44. The method according to Claim 43 wherein said preferential expression occurs from about -14 to about 25 days after pollination.
45. The method according to Claim 43 wherein said preferential expression occurs from about 4 to about 21 days after pollination.
46. The method according to Claim 43 wherein said preferential expression occurs from about 4 to about 12 days after pollination.
47. The method according to Claim 43 wherein said preferential expression occurs from about 8 to about 12 days after pollination.
48. (New) The method according to Claim 5 wherein said seed is from a dicotyledonous plant and said promoter is selected from the group consisting of bean β -phaseolin, napin, β -conglycinin and soybean lectin promoter.
49. (New) The method according to Claim 1 wherein the cytokinin modulating gene is selected from the group consisting of genes encoding cytokinin biosynthetic

Serial No. 09/545,334
Group Art Unit: 1635

Attorney Docket No. 0803

- enzymes, cytokinin catabolic enzymes, cytokinin catabolic enzyme antagonists and cytokinin biosynthetic enzyme agonists.
50. (New) The method according to Claim 49 wherein said modulating gene encodes a cytokinin catabolic enzyme.
51. (New) The method according to Claim 50 wherein said modulating gene encodes cytokinin oxidase.
52. (New) The plant according to Claim 18 wherein said seed is from a dicotyledonous plant and said promoter is selected from the group consisting of bean β -phaseolin, napin, β -conglycinin and soybean lectin promoter.
53. (New) The plant according to Claim 18 wherein the cytokinin modulating gene is selected from the group consisting of genes encoding cytokinin biosynthetic enzymes, cytokinin catabolic enzymes, cytokinin catabolic enzyme antagonists and cytokinin biosynthetic enzyme agonists.
54. (New) The plant according to Claim 53 wherein said modulating gene encodes a cytokinin catabolic enzyme.
55. (New) The plant according to Claim 54 wherein said modulating gene encodes cytokinin oxidase.
56. (New) The DNA according to Claim 30 wherein said seed is from a dicotyledonous plant and said promoter is selected from the group consisting of bean β -phaseolin, napin, β -conglycinin and soybean lectin promoter.
57. (New) The DNA according to Claim 30 wherein the cytokinin modulating gene is selected from the group consisting of genes encoding cytokinin biosynthetic enzymes, cytokinin catabolic enzymes, cytokinin catabolic enzyme antagonists and cytokinin biosynthetic enzyme agonists.
58. (New) The DNA according to Claim 57 wherein said modulating gene encodes a cytokinin catabolic enzyme.
59. (New) The DNA according to Claim 58 wherein said modulating gene encodes cytokinin oxidase.

Serial No. 09/545,334
Group Art Unit: 1635

REPLACEMENT PARAGRAPH FOR SPECIFICATION AT PAGES 28-29

C2

With respect to plants, examples of seed-preferred promoters include promoters of seed storage proteins which express these proteins in seeds in a highly regulated manner (Thompson, *et al.*; BioEssays: 10:108; (1989), incorporated herein in its entirety by reference), such as, for dicotyledonous plants, a bean β -phaseolin promoter, a napin promoter, a β -conglycinin promoter, and a soybean lectin promoter. For monocotyledonous plants, promoters useful in the practice of the invention include, but are not limited to, a maize 15 kD zein promoter, a 22 kD zein promoter, a 27Kd γ -zein promoter (such as gzw64A promoter, see Genbank Accession #S78780), a waxy promoter, a shrunken-1 promoter, a globulin 1 promoter (See Genbank Accession # L22344), an ltp2 promoter (Kalla, *et al.*, Plant Journal 6:849-860 (1994); U.S. Patent 5,525,716), cim1 promoter (see co-pending U.S. Patent application 09/377,648), and the shrunken-2 promoter. However, other promoters useful in the practice of the invention are known to those of skill in the art such as nucellain promoter (See C. Linnestad, *et al.*, *Nucellain, A Barley Homolog of the Dicot Vacuolar - Processing Proteasem Is Localized in Nucellar Cell Walls*, Plant Physiol. 118:1169-80 (1998), kn1 promoter (See S. Hake and N. Ori, *The Role of knotted1 in Meristem Functions*, B8: INTERACTIONS AND INTERSECTIONS IN PLANT PATHWAYS, COEUR D'ALENE, IDAHO, KEYSTONE SYMPOSIA, February 8-14, 1999, at 27.), and maize end 1 and end 2 promoters (See U.S. provisional patent application 60/098,230, filed August 28, 1998 and U.S. patent application 09/383,543, filed August 26, 1999). See also US patent applications 60/163,114, filed November 2, 1999, and 60/155,859, filed September 24, 1999. Spatially acting promoters such as glb1, an embryo-preferred promoter, or gamma zein, an endosperm-preferred promoter (such as BET1, See G. Hueros, *et al.*, *Molecular Characterization of BET-1, a Gene Expressed in the Endosperm Transfer Cells of Maize*, Plant Cell 7:747-57 (June 1995).), are particularly useful. The use of temporally acting promoters is also contemplated by this invention. Promoters that act from 0-25 days after pollination (DAP) are preferred, as are those acting from 4-21, 4-12, or 8-12 DAP. In this regard, promoters such as cim1 and ltp2 are preferred. Promoters that act from -14 to 0 days after pollination can also be used, such as SAG12 (See WO 96/29858, Richard M. Amasino, published 3 Oct. 1996.) and ZAG1 or ZAG2 (See R.J. Schmidt, *et al.*, *Identification and Molecular Characterization of ZAG1, the Maize Homolog of the Arabidopsis Floral Homeotic Gene AGAMOUS*, Plant-Cell 5(7): 729-37 (July 1993)).

Serial No. 09/545,334

VERSION MARKED TO SHOW CHANGES MADE

REPLACEMENT PARAGRAPH FOR SPECIFICATION AT PAGES 28-29

With respect to plants, examples of seed-preferred promoters include promoters of seed storage proteins which express these proteins in seeds in a highly regulated manner (Thompson, *et al.*; BioEssays, 10:108; (1989), incorporated herein in its entirety by reference), such as, for dicotyledonous plants, a bean β -phaseolin promoter, a napin promoter, a β -conglycinin promoter, and a soybean lectin promoter. For monocotyledonous plants, promoters useful in the practice of the invention include, but are not limited to, a maize 15 kD zein promoter, a 22 kD zein promoter, a 27Kd γ -zein promoter (such as gzw64A promoter, see Genbank Accession #S78780), a waxy promoter, a shrunken-1 promoter, a globulin 1 promoter (See Genbank Accession # L22344), an ltp2 promoter (Kalla, *et al.*, Plant Journal 6:849-860 (1994); U.S. Patent 5,525,716), cim1 promoter (see co-pending U.S. Patent application 09/377,648), and the shrunken-2 promoter. However, other promoters useful in the practice of the invention are known to those of skill in the art such as nucellain promoter (See C. Linnestad, *et al.*, *Nucellain, A Barley Homolog of the Dicot Vacuolar - Processing Proteasem Is Localized in Nucellar Cell Walls*, Plant Physiol. 118:1169-80 (1998), kn1 promoter (See S. Hake and N. Ori, *The Role of knotted1 in Meristem Functions*, B8: INTERACTIONS AND INTERSECTIONS IN PLANT PATHWAYS, COEUR D'ALENE, IDAHO, KEYSTONE SYMPOSIA, February 8-14, 1999, at 27.), and maize end 1 and end 2 promoters (See U.S. provisional patent application 60/098,230, filed August 28, 1998 and U.S. patent application 09/383,543, filed August 26, 1999). See also US patent applications 60/163,114, filed November 2, 1999, and 60/155,859, filed September 24, 1999. Spatially acting promoters such as glb1, an embryo-preferred promoter, or gamma zein, an endosperm-preferred promoter (such as BET1, See G. Hueros, *et al.*, *Molecular Characterization of BET-1, a Gene Expressed in the Endosperm Transfer Cells of Maize*, Plant Cell 7:747-57 (June 1995).), are particularly useful. The use of temporally acting promoters is also contemplated by this invention. Promoters that act from 0-25 days after pollination (DAP) are preferred, as are those acting from 4-21, 4-12, or 8-12 DAP. In this regard, promoters such as cim1 and ltp2 are preferred. Promoters that act from -14 to 0 days after pollination can also be used, such as SAG12 (See WO 96/29858, Richard M. Amasino, published 3 Oct. 1996.) and ZAG1 or ZAG2 (See R.J. Schmidt, *et al.*, *Identification and Molecular Characterization of ZAG1, the Maize Homolog of the Arabidopsis Floral Homeotic Gene AGAMOUS*, Plant-Cell 5(7): 729-37 (July 1993)).